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MOLECULAR MODELING OF 5-[(AMIDOBENZYL)OXY]-NICOTINAMIDES AS SIRTUIN 2 INHIBITORS USING ALIGNMENT-(IN)DEPENDENT 3D-QSAR ANALYSIS AND MOLECULAR DOCKING

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Abstract:

The group of 5-[(amidobenzyl)oxy]-nicotinamides represents promising group of sirtuin 2 (SIRT2) inhibitors. Despite structural similarity, representatives of this group of inhibitors displayed versatile mechanisms of inhibition which hamper rational drug design. The aim of this research was to form a 3D-QSAR (3D-Quantitative Structure-Activity Relationship) model, define the pharmacophore of this subgroup of SIRT2 inhibitors, define the mode of protein-ligand interactions and design new compounds with improved predicted activity and pharmacokinetics. For the 3D-QSAR study, data set was generated using structures and activities of 166 5-[(amidobenzyl)oxy]-nicotinamides. 3D-conformations of compounds were optimized, alignment-independent GRIND2 descriptors were calculated and 3D-QSAR PLS models were generated using 70% of data set. To investigate bioactive conformations of inhibitors, molecular docking was used. Molecular docking analysis identified two clusters of predicted bioactive conformations which is in alignment with experimental observations. The defined pharmacophoric features were used to design novel inhibitors with improved predicted potency and ADMET profiles.

Key words: 3D-QSAR, molecular docking, SIRT2 inhibitors, pharmacophore, ADMET

1. Introduction

Histon deacetylases (HDACs) are highly conserved class of epigenetic enzymes involved in regulation of post-translational modifications of lysine residues of many proteins. Class III of HDACs, known as sirtuins, represents deacetylases with nicotinamide adenine dinucleotide (NAD^+)-dependent catalytic mechanism. Sirtuin family consists of 7 isoforms (SIRT1-7) sharing conserved catalytic and NAD^+-binding domains. Sirtuin 2 (SIRT2) is the only sirtuin isoform with predominant residence in cytoplasm. Through protein-protein interactions, SIRT2 regulates post-translational modifications of many proteins crucial for maintenance of cell cycle and metabolism, including histones, various transcriptional factors, alpha-tubulin etc. Inhibition of SIRT2 emerged as promising strategy to combat cancer, metabolic diseases, as well as neurodegenerative diseases [1].

Despite therapeutic potential, none of SIRT2 inhibitors reached market mostly due to limited selectivity, potency and/or pharmacokinetic properties. Many chemotypes of SIRT2 inhibitors have been described so far [2]. Some of the most promising are derivatives of 5-

[(amidobenzyl)oxy]-nicotinamides. Despite sharing the same scaffold, mechanistic studies revealed that some 5-[(amidobenzyl)oxy]-nicotinamides have different mechanisms of action which could be either competitive or noncompetitive for NAD^+ and peptide substrate, implicating different binding modes of inhibitors [3].

Unknown binding mode and multiple binding modes within the same group of compounds could significantly hamper rational design of novel inhibitors. Considering the fact that structure of the complex of SIRT2 with 5-[(amidobenzyl)oxy]-nicotinamides has not been experimentally resolved yet, ligand-based molecular modelling techniques, such as 3D-quantitative structure-activity relationship (3D-QSAR), could aid rational design of novel inhibitors with improved characteristics. Although X-ray structures of SIRT2 in complex with other classes of inhibitors are known, detected conformational flexibility of SIRT2 urge for thoughtful validation of those structures before application of structure-based modelling techniques (as molecular docking) on 5-[(amidobenzyl)oxy]-nicotinamides.

The aim of this research was to provide rationale for the design of novel SIRT2 inhibitors through establishing 3D-QSAR models for prediction of SIRT2 inhibitors potency. Additionally, molecular docking analysis was performed in order to probe suitability of available SIRT2:inhibitor X-ray structures as starting structures for prediction of 5-[(amidobenzyl)oxy]-nicotinamides binding modes.

2. Materials and Methods

Initial dataset of 166 SIRT2 inhibitors was acquired from ChEMBL database (https://www.ebi.ac.uk/chembl/). Range of the inhibitory activity (expressed as pIC50 values) of compounds from dataset was 4.82 – 7.87. Protonation states of compounds were determined using MarvinSketch 6.1.0 software (https://chemaxon.com/products/marvin). Geometry optimization was performed using Hartree-Fock/3-21G* basis set in Gaussian 98W software (https://gaussian.com/). Data set was split into training (70% of compounds) and test set (30% of compounds). GRIND molecular descriptors were calculated measuring distances between different hot-spots of GRID force-field potential generated as molecular interaction field (MIF) surface around each molecule using Pentacle 1.0.7 software (https://www.moldiscovery.com/). Partial last squares (PLS) method was used for modelling the correlation between structure descriptors and activities. Quality of the models has been estimated using correlation coefficient (R^2), correlation coefficient of leave-one-out cross-validation (Q^2), R^2 of prediction (R^2_{pred}) and standard-errors of prediction (SDEP). ADMET predictor software (https://www.simulations-plus.com/software/admetpredictor/) was used to predict pharmacokinetic features of designed compounds.

Protein-ligand complex for molecular docking was obtained from Protein Data Bank PDB IDs: 5y5n, 5yqn, 5dy4. Molecular docking was performed using AutoDock Vina (http://vina.scripps.edu/) and free energy of binding was predicted using default scoring function of Vina. AutoLigand module was used to define search space around ligand in binding pocket. Ten top-active compounds from data set have been used for docking.

3. Results and Discussion

External and internal validation parameters of the created 3D-QSAR model (Table 1) indicated good performance of the model and justify its usage for the rational design of structurally related compounds. Created model consisted of two latent variables and 300 descriptors. Although GRIND descriptors are alignment independent by definition, our preliminary results indicated strong dependence of 3D-QSAR model on the initial 3D-conformation. Analysis of the PLS-coefficients revealed the most important structural features required for activity and allowed definition of GRIND-based pharmacophoric features. In Fig. 1, the most important descriptors are exemplified on one of the top active compound from training set. Positive PLS coefficients revealed two acceptors of hydrogen bond at the distances of 17.2-17.6 Å (Var. 183) and 16.8-17.2 (Var. 182), as being important for inhibitors activity. Additionally, model revealed presence of hydrogen bond acceptor and steric feature at the

distance of 21.2-21.6 Å (Var. 683), as well as presence of hydrogen bond donor and steric feature at the distance of 22.2-22.4 Å (Var. 615) as important for activity (Fig. 1). On the other hand, negative PLS coefficients pointed to GRIND-based pharmacophoric features related to decrease of inhibitory activity such as presence of hydrogen bond donor and steric feature at the distance of 17.6-18.0 Å (Var. 604), as well as hydrogen bond donor and acceptors at the distance of 15.6-16.0 Å (Var. 529) (Fig. 1).

Table 1. Results of 3D-QSAR model validation.

Parameter	Training set	Test set	Limits
R^2	0.81	/	>0.8
Q^2	0.67	/	>0.5
R^2_{pred}	/	0.55	>0.5
SDEP	0.41	0.40	/

Based on the defined pharmacophoric features, novel inhibitors had been designed and their activity was predicted using validated 3D-QSAR model. Since the most of the positively related descriptors point at nicotinamide portion of the inhibitor, this part of the structure in the design was kept constant. Additionally, predicted ADMET properties of the designed inhibitors were compared to the most active ligands, and only inhibitors with improved pharmacokinetics were retained for future studies.

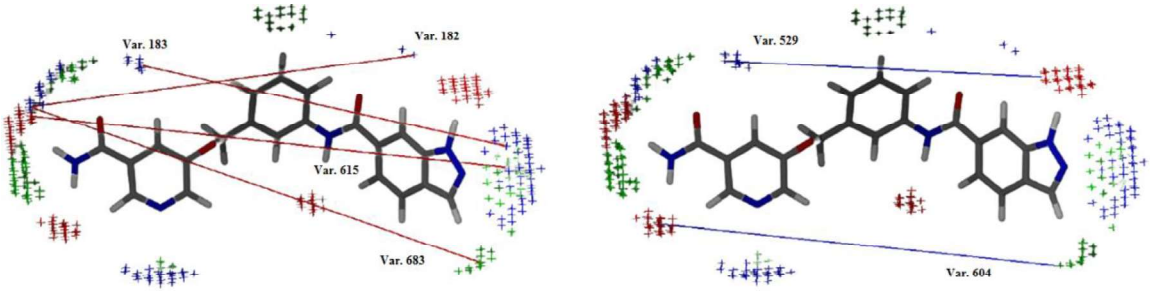

Fig. 1. Representation of obtained GRIND-based pharmacophore. Left – variables/GRIND descriptors with the highest PLS coefficients (red lines). Right – variables/GRIND descriptors with the lowest PLS coefficients (blue lines). MIF fields are represented as dots (blue – hydrogen bond acceptor; red – hydrogen bond donor; green – steric feature).

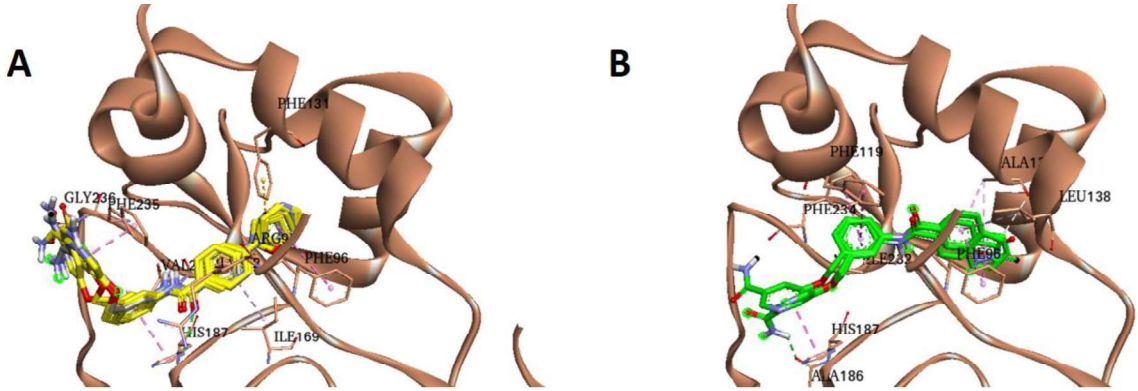

Fig. 2. Results of molecular docking. A – first, the most populated, cluster of predicted bioactive conformations. B – second cluster of bioactive conformations.

In order to investigate binding modes of 5-[(amidobenzyl)oxy]-nicotinamides, molecular docking was performed on 10 top-active inhibitors from data set. Considering the experimental observation of SIRT2 conformational flexibility, three different X-ray structures of SIRT2 in different conformational states were probed. According to our results, the most consistent docking poses were obtained when PDB ID:5y5n structure was used for molecular docking.

Molecular docking identified existence of two different clusters of bioactive conformations (Fig. 2). In both clusters, nicotinamide portion of the ligands was oriented towards solvent-exposed surface of binding pocket. Compounds of cluster 2 were able to enter deeper inside binding pocket. Existence of two slightly different binding modes is in accordance with experimental observation of different mechanisms of inhibition within the group of 5-[(amidobenzyl)oxy]-nicotinamides.

4. Conclusions

In this study, 3D-QSAR model for prediction of potency of 5-[(amidobenzyl)oxy]-nicotinamides as SIRT2 inhibitors was created and validated. Statistical parameters indicated good predictive power of trained model. Pharmacophoric features extracted from the PLS coefficients of the model were used in the rational design of novel representatives with improved properties. Additionally, molecular docking was used to provide insight into binding mode of this group of inhibitors and generate starting points for future structure-based design studies.

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